

NEW ACRIMARINES FROM CITRUS PLANTS<sup>1</sup>

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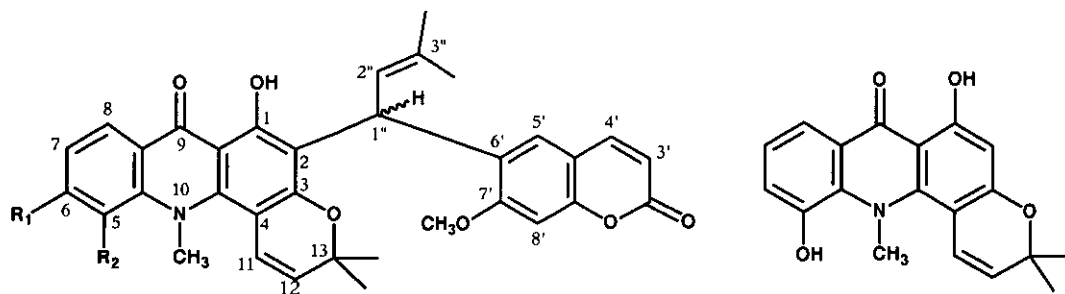
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Abstracts — Five new acrimarines (acridone-  
coumarin dimers) were isolated from some Citrus  
plants (Rutaceae) and their structures were  
elucidated by spectroscopic method.

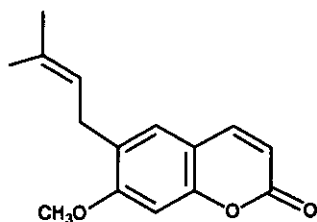
Recently, we reported the isolation and structure elucidation<sup>2,3</sup> of  
acrimarine-A ~ -H, dimeric compounds in which an acridone unit has been  
coupled to a coumarin. Further examination of the roots of "Yalaha"  
[several hybrid seedlings resulting from a cross of Duncan grapefruit  
(Citrus paradisi Macf.) x Dancy tangerine (C. tangerine Hort. ex Tanaka)]  
and "Funadoko" (C. funadoko Hort. ex Tanaka) afforded five new compounds,  
named acrimarine-I (1), -J (4), -K (5), -L (6) and -M (7) and in this  
paper we wish to report the isolation and structure elucidation of these  
new compounds.

Acrimarine-I (1) was obtained as yellow oil,  $[\alpha]_D +27.8^{\circ}(\text{CHCl}_3)$ . The high resolution ms showed molecular formula  $\text{C}_{34}\text{H}_{31}\text{NO}_7$ , which was considered to give rise to two characteristic ions from the halves of the molecule at  $m/z$  323 and 242 corresponding to formulae  $\text{C}_{19}\text{H}_{17}\text{NO}_4$  and  $\text{C}_{15}\text{H}_{14}\text{O}_3$ , respectively, indicating the presence of acridone and coumarin structures. The ir (3400, 1720, 1620, 1560  $\text{cm}^{-1}$ ) and uv [ 205, 223 (sh), 270 (sh), 296, 328, 348 (sh) nm] spectra suggested the presence of 1-hydroxy-9-acridone moiety.<sup>4</sup> The  $^1\text{H}$  nmr spectrum showed the presence of hydrogen bonded hydroxyl group ( $\delta$  14.63), ABC type aromatic protons [ $\delta$  7.94 (1H, dd,  $J=7.9, 1.8$  Hz), 7.15 (1H, t,  $J=7.9$  Hz), 7.09 (1H, dd,  $J=7.9, 1.8$  Hz)], 1,1-dimethylpyran ring [ $\delta$  6.61 (1H, d,  $J=9.8$  Hz), 5.48 (1H, d,  $J=9.8$  Hz), 1.14, 1.46 (each 3H, s)], characteristic H-4', H-3', H-5' and H-8' of coumarin nucleus [ $\delta$  7.67, 6.22 (each 1H, d,  $J=9.2$  Hz), 7.62, 6.69 (each 1H, s)], and a prenyl group connected with two aryl moieties [ $\delta$  5.94 (1H, d,  $J=9.8$  Hz), 5.74 (1H, d,  $J=9.8$  Hz), 1.80 (3H, s), 1.75 (3H, s)]. Two singlets at  $\delta$  3.75 and 3.72 in the  $^1\text{H}$  nmr and two quartets at  $\delta$  55.9 and 48.5 in the  $^{13}\text{C}$  nmr spectra showed the presence of each one methoxy and N-methyl group. In nOe experiments, irradiation of the methoxy signal at  $\delta$  3.75 gave 20% increments of the signal at  $\delta$  6.69 (H-8'). On the other hand, when the N-methyl signal at  $\delta$  3.72 was irradiated, 13% increments were observed on the signal at  $\delta$  6.61 (H-11). From these results, the location of methoxy group was assigned to C-7' and the angular orientation of the dimethylpyran ring was confirmed. The similarities of signal patterns of  $^1\text{H}$  nmr spectrum suggested the presence of 5-hydroxynoracronycine (2)<sup>5</sup> and suberosin (3)<sup>6</sup> moieties in the structure. The location of the suberosin unit was elucidated by  $^1\text{H}$  detected heteronuclear multiple bond connectivity (HMBC) spectrum. The H-bonded proton signal at  $\delta$  14.63 showed long-range correlations with the

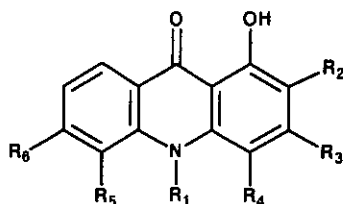


- (1)  $R_1=H, R_2=OH$   
 (4)  $R_1=OH, R_2=OCH_3$

(2)



(3)



- (5)  $R_1=CH_3, R_2=Cou, R_3=OH, R_4=H, R_5=OCH_3, R_6=OH$   
 (6)  $R_1=R_2=H, R_3=OCH_3, R_4=Cou, R_5=OCH_3, R_6=OH$   
 (7)  $R_1=CH_3, R_2=Cou, R_3=OH, R_4=R_5=R_6=H$

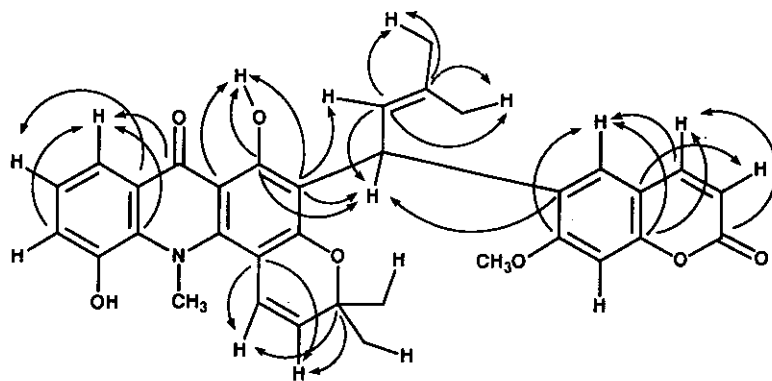
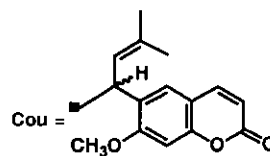


Figure 1

C-H Correlations in the HMBC spectrum ( $J=8$  Hz) of acrimarine-I (1)

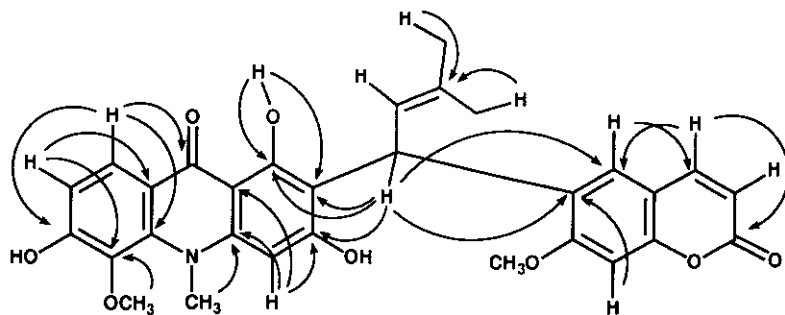


Figure 2

H-C Correlations in H-C long range COSY spectrum ( $J=5$  Hz) of acrimarine-K (5)

carbon signals at  $\delta$  162.0 (C-1), 112.6 (C-2), and 107.1 (C-9a). Further, the proton signal at  $\delta$  5.74 (H-1'') was correlated with carbon signals at  $\delta$  162.0 (C-1), 112.6 (C-2), 159.0 (C-3), 132.9 (C-3''), 128.6 (C-5'), and 130.6 (C-6'). Other CH long range correlations observed are shown by arrows in Figure 1. Based on these spectral data, the structure of acrimarine-I was concluded to 1.

Acrimarine-J (4) was obtained as yellow oil,  $[\alpha]_D \pm 0^\circ$  (CHCl<sub>3</sub>). The mass spectrum showed the molecular ion at  $m/z$  595 which corresponded to C<sub>35</sub>H<sub>33</sub>NO<sub>8</sub>, a difference of CH<sub>2</sub>O compared with 1. The <sup>1</sup>H nmr features were similar to those of 1, except for appearances of one more methoxy ( $\delta$  3.90) signal and change of ABC type aromatic protons to AB type [ $\delta$  7.96, 6.97 (each 1H, d, J= 8.5 Hz)]. The signals at  $\delta$  3.90, 3.84, 3.75 in the <sup>1</sup>H nmr and  $\delta$  60.5, 56.5, 48.9 in the <sup>13</sup>C nmr spectra indicated the presence of two methoxy and one N-methyl group. The location of methoxy groups and the situation of 1,1-dimethylpyran ring were determined by nOe experiments. Irradiation of the signal at  $\delta$  3.75 and 3.84 showed 14% and 20% increments of the signals at  $\delta$  6.69 (H-11) and 6.83 (H-8'), respectively. When the signal at  $\delta$  3.90 was irradiated, no increments were observed on any signals. These results indicated the locations of methoxy groups at C-5 and C-7', phenolic hydroxyl group at C-6, and the angular situation of 1,1-dimethylpyran ring. On the basis of these results, structure (4) was assigned to acrimarine-J.

Acrimarine-K (5) was isolated as yellow oil,  $[\alpha]_D \pm 0^\circ$  (CHCl<sub>3</sub>). The molecular formula C<sub>30</sub>H<sub>27</sub>NO<sub>8</sub> was established by high resolution ms. The <sup>1</sup>H nmr spectrum showed the signals of ortho coupled [ $\delta$  8.04, 6.94 (each 1H, d, J= 8.8 Hz)], isolated [ $\delta$  6.48 (1H, s)] aromatic protons, characteristic signals due to H-4', H-3', H-5' and H-8' of coumarin nucleus [ $\delta$  7.88, 6.16 (each 1H, d, J= 9.5 Hz), 7.73, 6.82 (each 1H, s)].

and signals due to a prenyl group coupled to two aryl groups [ $\delta$  1.75 (3H, s), 1.72 (3H, s), 5.83 (1H, d,  $J=9.5$  Hz) and 6.08 (1H, d,  $J=9.5$  Hz)]. The signals at  $\delta$  3.98, 3.87, 3.77 in  $^1\text{H}$  nmr and  $\delta$  40.2, 56.9, 62.2 in  $^{13}\text{C}$  nmr spectra indicated the presence of two methoxy and one N-methyl group. The location of methoxy groups was determined by nOe experiments. Irradiation of the signal at  $\delta$  3.98 and 3.87 showed 14% and 16% increments of the signal at  $\delta$  6.48 (H-4) and 6.82 (H-8'), respectively. On the other hands, when the signal at  $\delta$  3.77 was irradiated, no increments were observed at any proton signals. Thus, the location of two methoxy groups was determined to C-5 and C-7'. From the above data, the location of suberosin unit was assigned at C-2 in the acridone skeleton, and the structure of acrimarine-K was determined to 5. The proposed structure was further confirmed by HC long range COSY shown by arrows in Figure 2. Acrimarine-L (6) was isolated as yellow oil,  $[\alpha]_D^{20}$  (CHCl<sub>3</sub>), and gave a molecular ion at  $m/z$  529 which corresponded to C<sub>30</sub>H<sub>27</sub>NO<sub>8</sub>, the same as that of acrimarine-K (5). The  $^1\text{H}$  nmr spectrum showed a good similarity with that of 5, except for small differences of chemical shifts indicating the presence of suberosin and 1,3,5,6-oxygenated 9-acridone units. The signals at  $\delta$  3.97, 3.94 and 3.87 indicated the presence of methoxy groups. In the nOe experiments, irradiation of the signal at  $\delta$  3.97 and 3.87 showed 17% and 14% increments of the signal at  $\delta$  6.34 (H-2) and 6.92 (H-8'), respectively. On the other hands, when the signal at  $\delta$  3.94 was irradiated, no increments were observed. Meanwhile, when the signal at  $\delta$  9.14 assumed to NH signal was irradiated, 12% and 9% increments were observed on the signal at  $\delta$  5.78 (H-1'') and 5.91 (H-2''), respectively. These results indicated the locations of methoxy groups were at C-3, C-5 and C-7', and the linking positions of acridone and coumarin rings were at C-4 and C-6', respectively. The above data led us to assign structure

6 to acrimarine-L.

Acrimarine-M (7) was isolated as yellow oil,  $[\alpha]_D \pm 0^\circ$  ( $\text{CHCl}_3$ ). The molecular formula,  $\text{C}_{29}\text{H}_{25}\text{NO}_6$ , was confirmed by high resolution ms. The  $^1\text{H}$  nmr spectrum showed, besides signals of suberosin unit, ABCD type [ $\delta$  8.38 (1H, d,  $J= 8.1$  Hz), 7.76 (1H, t,  $J= 8.1$  Hz), 7.73 (1H, d,  $J= 8.1$  Hz), 7.30 (1H, t,  $J= 8.1$  Hz)] and a lone [ $\delta$  6.54 (1H, s)] aromatic proton signal and two methyl singlets ( $\delta$  3.82 and 3.86). The location of two methyl signals at  $\delta$  3.86 and 3.82 was assigned by nOe experiments. When the signal at  $\delta$  3.82 was irradiated, each 14% and 10% increments were observed on the signals at  $\delta$  7.73 (H-5) and 6.54 (H-4), respectively. On the other hands, irradiation of the signal at  $\delta$  3.86 showed 11% increments of the signal at  $\delta$  6.82 (H-8'). The above data showed the presence of N-methyl group, the location of methoxy group at C-7', and the coupling position of the acridone and the coumarin nuclei was at C-2 and C-6'. From these results, the structure 7 was assigned to acrimarine-M. The absolute configurations of these new acrimarines remain to be determined.

#### EXPERIMENTAL

Extraction and Isolation of Acrimarine-I (1) and -J (4) The roots of "Yalaha" [several hybrid seedlings resulting from a cross of Duncan grapefruit (*C. paradisi* Macf.) x Dancy tangerine (*C. tangerine* Hort. ex Tanaka)] (750 g) collected at the orchard of Okitsu Branch, Fruit Tree Research Station was extracted with acetone (2 L x 2) under reflux for 8hr. The extract (103.1 g) was chromatographed over silica gel column and eluted with toluene, toluene- $\text{CH}_2\text{Cl}_2$ ,  $\text{CH}_2\text{Cl}_2$ , acetone- $\text{CH}_2\text{Cl}_2$ , acetone and MeOH. The acetone- $\text{CH}_2\text{Cl}_2$  (1:9) eluate was further separated by silica gel column, centrifugal chromatography, PTLC developed with acetone- $\text{CHCl}_3$

(1:9), MeOH-CHCl<sub>3</sub> (1:19), acetone-benzene (2:8) to give acrimarine-I (2.3 mg) and -J (5.3 mg) together with many other compounds.

Isolation of Acrimarine-K (5), -L (6) and -M (7) The AcOEt eluate of silica gel column chromatography of acetone extracts of *C. funadoko* Hort. ex Tanaka reported before<sup>2</sup> was further separated by PTLC using appropriate solvent systems of isopropyl ether, acetone-CHCl<sub>3</sub>(1:9), acetone-benzene (2:8), MeOH-CHCl<sub>3</sub> (1:19), AcOEt-benzene (3:7) to give acrimarine-K (13.7 mg), -L (3.2 mg) and -M (2.1 mg).

Acrimarine-I (1) Yellow oil,  $[\alpha]_D^{26} +27.8^{\circ}$  (c= 0.036, CHCl<sub>3</sub>); uv  $\lambda_{max}$  (EtOH): 205, 223(sh), 270 (sh), 296, 328, 348 (sh) nm; ir (CHCl<sub>3</sub>) 3400, 1720, 1620, 1560 cm<sup>-1</sup>; hrms m/z 565.2115 [M]<sup>+</sup> (C<sub>34</sub>H<sub>31</sub>NO<sub>7</sub> calcd 565.2101), m/z 323.1135 [(C<sub>19</sub>H<sub>17</sub>NO<sub>4</sub>)<sup>+</sup>, calcd 323.1158], m/z 242.0950 [(C<sub>15</sub>H<sub>14</sub>O<sub>3</sub>)<sup>+</sup>, calcd 242.0943]; eims m/z: 565 (M<sup>+</sup>, base peak), 550, 522, 496, 380, 365, 312, 297.

Acrimarine-J (4) Yellow oil,  $[\alpha]_D^{28} \pm 0^{\circ}$  (c= 0.106, CHCl<sub>3</sub>); uv  $\lambda_{max}$  (EtOH) 205, 226 (sh), 271, 287, 304 (sh), 343 nm; ir (CHCl<sub>3</sub>) 3440, 1720, 1620, 1560 cm<sup>-1</sup>; hrms m/z 595.2209 [M]<sup>+</sup> (C<sub>35</sub>H<sub>33</sub>NO<sub>8</sub> calcd 595.2206); eims m/z 595 (M<sup>+</sup>, base peak), 580, 552, 526, 484, 406, 354, 331, 278, 242.

Acrimarine-K (5) Yellow oil,  $[\alpha]_D^{25} \pm 0^{\circ}$  (c= 0.0695, CHCl<sub>3</sub>); uv  $\lambda_{max}$  (MeOH) 205, 219, 263, 276, 300 (sh), 331 nm; ir (CHCl<sub>3</sub>) 3500, 3400 (br), 1720, 1620, 1600, 1570 cm<sup>-1</sup>; hrms m/z 529.1751 [M]<sup>+</sup> (C<sub>30</sub>H<sub>27</sub>NO<sub>8</sub> calcd 529.1734), m/z 243.0980 [(C<sub>15</sub>H<sub>15</sub>O<sub>3</sub>)<sup>+</sup>, calcd 243.1019], m/z 287.0826 [(C<sub>15</sub>H<sub>13</sub>NO<sub>5</sub>)<sup>+</sup>, calcd 287.0793]; eims m/z 529 (M<sup>+</sup>), 486, 288, 287 (base peak), 273, 272, 244, 243, 242, 230, 228, 227, 214, 213, 212, 211.

Acrimarine-L (6) Yellow oil,  $[\alpha]_D^{25} \pm 0^{\circ}$  (c= 0.061, CHCl<sub>3</sub>); uv  $\lambda_{max}$  (MeOH) 205, 221, 256, 266, 286 (sh), 295 (sh), 328 nm; ir (CHCl<sub>3</sub>) 3380 (br), 1730, 1640, 1620, 1570 cm<sup>-1</sup>; hrms m/z 529.1730 [M]<sup>+</sup> (C<sub>30</sub>H<sub>27</sub>NO<sub>8</sub> calcd 529.1734), m/z 242.0918 [(C<sub>15</sub>H<sub>14</sub>O<sub>3</sub>)<sup>+</sup>, calcd 242.0942], m/z 287.0751

Table.  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectral data of acrimarinines (acetone- $\text{d}_6$ )

	1*		4		5		6		7	
	$\delta\text{H}$	$\delta\text{C}$	$\delta\text{H}$	$\delta\text{C}$	$\delta\text{H}$	$\delta\text{C}$	$\delta\text{H}$	$\delta\text{C}$	$\delta\text{H}$	$\delta\text{C}$
1-OH	14.63	162.0	15.07	162.8	15.26	164.1	14.65		15.42	
2		112.6		112.3		105.5	6.34			
3		159.0		161.8		163.6				
3-Ome							3.97			
4		102.4		103.4	6.48	92.8			6.54	
4a		146.2		146.8		147.0				
5		146.3		143.5		136.6			7.73 d (8.1)	
5-Ome			3.90	60.5	3.77	62.2	3.94			
6	7.09 dd (7.9, 1.8)	120.0		159.4		157.3			7.76 t (8.1)	
7	7.15 t (7.9)	123.0	6.97 d (8.5)	113.8	6.94 d (8.8)	113.3	6.88 d (8.7)		7.30 t (8.1)	
8	7.94 dd (7.9, 1.8)	118.6	7.96 d (8.5)	124.9	8.04 d (8.8)	124.2	7.84 d (8.7)		8.38 d (8.1)	
8a		125.6		118.6		117.9				
9		182.1		182.4		181.3				
9a		107.1		106.9		112.8				
10a		137.0		132.5		139.9				
N-Me	3.72	48.5	3.75	48.9	3.98	40.2			3.82	
N-H							9.14			
11	6.61 d (9.8)	121.3	6.69 d (9.8)	121.9						
12	5.48 d (9.8)	123.6	5.81 d (9.8)	123.3						
13		76.4		77.3						
13-Me	1.14	27.3	1.50	26.1						
	1.46	32.7	1.50	27.4						
2'		161.9		161.3		161.8				
3'	6.22 d (9.2)	112.4	6.17 d (9.8)	113.2	6.16 d (9.5)	113.5	6.21 d (9.7)		6.15 d (9.5)	
4'	7.67 d (9.2)	144.2	7.92 d (9.8)	145.0	7.88 d (9.5)	145.6	7.98 d (9.7)		7.89 d (9.5)	
4a'		111.5		113.3		112.8				
5'	7.62	128.6	7.76	129.7	7.73	130.0	7.78		7.74	
6'		130.6		131.0		111.7				
7'		160.9		159.4		162.3				
7'-Ome	3.75	55.9	3.84	56.5	3.87	56.9	3.87		3.86	
8'	6.69	98.4	6.83	99.2	6.82	99.6	6.92		6.82	
8'a		154.3		155.4		155.8				
1''	5.74 d (9.8)	26.4	5.63 d (9.8)	33.5	5.83 d (9.5)	34.1	5.78 d (8.4)		5.83 d (9.5)	
2''	5.94 d (9.8)	124.1	6.01 d (9.8)	125.8	6.08 d (9.5)	126.7	5.91 d (8.4)		6.09 d (9.5)	
3''		132.9		132.5		131.3				
3''-Me	1.75	18.1	1.74	18.2	1.72	18.7	1.66		1.73	
	1.80	26.1	1.76	26.6	1.75	26.5	1.82		1.75	

Values are given in  $\delta$  ppm. Figures in parentheses are coupling constant (J) in Hz. \* Measured in  $\text{CDCl}_3$ .



$[(C_{15}H_{13}NO_5)^+]$ , calcd 287.0792]; eims  $m/z$  529 ( $M^+$ ), 301, 288, 287, 272, 244, 243, 242, 229, 228, 227, 214, 213 (base peak), 212, 211.

$^{13}C$  nmr (acetone- $d_6$ )  $\delta$  57.0, 57.2, 61.5.<sup>7</sup>

Acrimarine-M (7) Yellow oil.  $[\alpha]_D^{25} \pm 0^0$  ( $c = 0.052$ ,  $CHCl_3$ ); uv  $\lambda_{max}$  (MeOH) 205, 224, 248, 277, 296 (sh), 331 nm; ir ( $CHCl_3$ ) 3400 (br), 1725, 1620, 1600  $cm^{-1}$ ; hrms  $m/z$  483.1707 [ $M^+$ ] ( $C_{29}H_{25}NO_6$  calcd 483.1681),  $m/z$  241.0735  $[(C_{14}H_{11}NO_3)^+]$ , calcd 241.0737],  $m/z$  242.0979  $[(C_{15}H_{14}O_3)^+]$ , calcd 242.0942]; eims  $m/z$  483 ( $M^+$ ), 441, 440 (base peak), 396, 294, 292, 266, 254, 243, 242, 241, 228, 227, 226, 213, 212, 211.

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7. Because of the small quantity of the sample available, it was impossible to detect the other signals.

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